

Clinical implications of neutrophil-to-lymphocyte ratio and MDSC kinetics in gastric cancer patients treated with ramucirumab plus paclitaxel

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Abstract

Objective: We aimed to investigate the prognostic value of neutrophil-to-lymphocyte ratio (NLR) and myeloid-derived suppressor cells (MDSCs) in gastric cancer patients treated with second-line ramucirumab plus paclitaxel.

Methods: A total of 116 patients with advanced or metastatic gastric cancer who receive ramucirumab plus paclitaxel were prospectively enrolled. Fresh blood samples were collected before and after treatment, and flow cytometry was performed to assess the proportions of monocytic (mMDSCs) and granulocytic MDSCs (gMDSCs).

Results: Median age was 58 years and 71 (61.2%) patients were male. A baseline $NLR \geq 2.94$ was associated with significantly poorer progression-free survival (PFS) and overall survival (OS) vs. an $NLR < 2.94$ ($P=0.011$ and $P=0.002$, respectively). In multivariate analysis, an $NLR \geq 2.94$ was independently associated with poorer PFS [hazard ratio (HR)=1.58; 95% confidence interval (95% CI): 1.01–2.49, $P=0.046$] and OS (HR=1.77; 95% CI: 1.04–3.04, $P=0.036$). While mMDSC counts did not significantly change following two cycles of therapy ($P=0.530$), gMDSC counts decreased significantly after two treatment cycles ($P=0.025$) but tended to increase in patients with progressive disease after two treatment cycles ($P=0.098$). A progressive increase in gMDSC counts ($\geq 44\%$) was associated with a significantly shorter PFS and OS vs. a gMDSC count increase $< 44\%$ ($P=0.001$ and $P=0.003$, respectively).

Conclusions: The baseline NLR may help guide clinical decisions during ramucirumab plus paclitaxel therapy for gastric cancer. Our gMDSC kinetics data warrant further clinical validation and mechanistic investigation.

Keywords: Gastric cancer; ramucirumab plus paclitaxel; neutrophil-to-lymphocyte ratio; myeloid-derived suppressor cells

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Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related death worldwide (1). Patients with advanced or metastatic gastric cancer have a poor prognosis and pose significant socioeconomic burdens, especially in Asian countries. Pivotal phase 3 trials

have established the role of second-line systemic chemotherapy for patients with disease progression despite platinum and fluoropyrimidine-based first-line systemic therapies, and these studies have demonstrated the superior efficacy of systemic therapy over best supportive care (2–4). Subsequently, the phase 3 Rainbow trial demonstrated the superior efficacy of the combination of anti-angiogenic

agent ramucirumab plus paclitaxel vs. paclitaxel monotherapy (5), which led to the designation of this combination as the current standard of second-line care. Although a recent exploratory analysis of the Rainbow trial has suggested the prognostic value of some circulating factors (6), the mechanism underlying the therapeutic response or resistance to ramucirumab plus paclitaxel remains poorly understood, and no predictive biomarker has been identified.

Investigations of systemic inflammatory response markers related to the clinical outcomes of gastric cancer patients are based on the concept that systemic inflammation may contribute to cancer progression through various mechanisms such as induction of cancer proliferation/metastasis, promotion of angiogenesis and attenuation of anti-tumor immune responses (7). Among these potential markers, neutrophil-to-lymphocyte ratio (NLR) was shown to be correlated well with the clinical outcomes of patients with gastric cancer who underwent surgical resection (8-10) or received palliative first-line (11-14) and second-line (15,16) systemic chemotherapy. A previous study suggested NLR as a prognostic factor in patients with gastric cancer who were treated with ramucirumab monotherapy or ramucirumab plus paclitaxel (17); however, inhomogeneous treatment regimen and lack of mechanistic explanation in that study warrant further validation of its clinical value and an investigation of biological features linked to NLR.

Myeloid-derived suppressor cells (MDSCs) are a population of immunosuppressive cells that play a critical role in immune evasion of cancers (18). Reports suggest that the proportion of MDSCs is correlated with NLR (19,20) and cancer stage (21) in patients with gastric cancer. Particularly, an inverse correlation between proportion of MDSCs and function of effector T cells has been observed in gastric cancer patients (22), and this highlights the role of MDSCs in attenuating anti-tumor immune response. Interestingly, both ramucirumab and paclitaxel can potentially modulate MDSCs. Vascular endothelial growth factor (VEGF) can induce the accumulation of aberrant myeloid cells via VEGF receptor 2 (VEGFR2) (23), and a positive correlation between VEGF score and MDSCs has been reported (20,24). Therefore, ramucirumab, a VEGFR2 antagonist, can theoretically modulate MDSCs by interrupting the VEGF/VEGFR2 pathway (25). Paclitaxel has also been shown to reduce MDSC counts (26) and promote differentiation of MDSCs into dendritic

cells (27) in preclinical models. However, mechanism by which ramucirumab plus paclitaxel combination therapy affects MDSCs and clinical implications in patients with gastric cancer have not yet been investigated.

Given the potential modulatory effects of ramucirumab and paclitaxel on MDSC count, we hypothesized that the kinetic changes of MDSCs and NLR might be associated with survival outcomes in gastric cancer patients receiving ramucirumab plus paclitaxel treatment. Therefore, we aimed to investigate the prognostic value of NLR and MDSC counts and their kinetics in a prospective cohort of gastric cancer patients treated with second-line ramucirumab plus paclitaxel.

Materials and methods

Study patients and ramucirumab plus paclitaxel therapy

This study was based on a prospective cohort of 116 patients with advanced or metastatic gastric cancer who received ramucirumab plus paclitaxel as a second-line systemic therapy between April 2018 and October 2019. Inclusion criteria were as follows: 1) age of 18 years or older; 2) receipt of ramucirumab plus paclitaxel therapy as second-line systemic therapy; 3) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; and 4) adequate blood sample quality for flow cytometry analysis. The Institutional Review Board approved the study protocol, and all patients provided written informed consent. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Ramucirumab (8 mg/kg IV on d 1 and 15) and paclitaxel (80 mg/m² IV on d 1, 8 and 15) were administered every 4 weeks until disease progression or unacceptable adverse events occurred. Study patients were followed up and examined every day of ramucirumab plus paclitaxel administration (d 1, 8, and 15 of each cycle). Complete blood cell counts and routine chemistry labs were measured on the follow-up day. Computed tomography (CT) scans were done every 8–12 weeks for disease evaluation. When disease progression was clinically suspected during follow-up, additional CT scans were performed. The study patients were additionally monitored by a study coordinator. The Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 were used to grade the tumor responses.

Calculation of NLR and immunophenotyping by flow cytometry

The NLR value was obtained by dividing absolute neutrophil count by absolute lymphocyte count. Both cell counts were based on complete blood analyses conducted routinely in clinical practice.

For immunophenotyping, blood samples were collected at baseline and after two cycles of ramucirumab plus paclitaxel therapy. Baseline blood samples were collected from all study patients (N=116), while follow-up samples were collected from 66 patients after two cycles of chemotherapy. Peripheral blood mononuclear cells were isolated from whole blood using Lymphoprep™ (Stem Cell Technologies) according to the manufacturer's instructions. No patients received granulocyte colony-stimulating factor within 2 weeks prior to NLR and MDSC evaluation.

Flow cytometry was performed using an S1000Exi (Stratedigm) or FACSLyric instrument (BD Biosciences, San Jose, CA, USA). Flow cytometry data were analyzed using FlowJo software (Treestar, San Carlos, USA). The following antibodies were used in the flow cytometry analyses: anti-CD33 (P67.6; BD Biosciences) and anti-HLA-DR (L243), anti-CD11b (M1/70), and anti-CD66b (G10F5) (all Biolegend, San Diego, CA, USA). Monocytic MDSCs (mMDSCs) were defined as HLA-DR⁻CD11b⁺CD33⁺CD66b⁻ cells, while granulocytic MDSCs (gMDSCs) were defined as HLA-DR⁻CD11b⁺CD33⁺CD66b⁺ cells. The MDSC counts were estimated from white blood cell counts and proportions of MDSC subsets in leukocyte counts. The representative flow cytometry gating strategy is presented in *Supplementary Figure S1*.

Statistical analysis

Progression-free survival (PFS) was defined as the interval from the initiation of ramucirumab plus paclitaxel (index date) to the date of disease progression (determined using RECIST v1.1) or death. Overall survival (OS) was defined as the interval between the index date and the date of death from any cause. The Kaplan-Meier method was used to estimate survival outcomes, and the log-rank test was used to compare these survival outcomes among the subgroups. The Chi-square test or Fisher's exact test was used to compare categorical variables among the subgroups. Cox proportional hazard modeling was used to assess the associations between examined factors and PFS and OS. In

addition to demographic factors and NLR, several prognostic factors shown to be associated with survival outcomes in the second-line setting, such as the time-to-progression (TTP) with first-line systemic therapy, ECOG performance status, lactate dehydrogenase (LDH) concentration, and hemoglobin concentration, were included in the multivariate analysis (15,28,29). The maximal Chi-square method was used to determine the optimal cut-off value of NLR and the percent change in the gMDSC count that best segregated PFS outcomes. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using R software (Version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria). The following R packages were used in this study: dplyr (Version 1.0.2), survival (Version 3.2.3), survminer (Version 0.4.8), maxstat (Version 0.7.25) and ggpubr (Version 0.4.0).

Results

Patient characteristics

Baseline characteristics of 116 patients included in this study are summarized in *Table 1*. The median age was 58 years, and 71 (61.2%) patients were male. Additionally, 108 (93.1%) and eight (6.9%) patients had ECOG performance scores of 0–1 or 2, respectively. The median TTP with first-line systemic therapy was 6.5 [interquartile range (IQR): 3.9–12.1] months. The median NLR and hemoglobin were 2.0 g/dL and 11.4 g/dL, respectively. The baseline mMDSC and gMDSC counts were 154.8/ μ L and 1,953.1/ μ L, respectively.

The median PFS and OS of study patients were 3.35 [95% confidence interval (95% CI): 3.29–4.47] months and 7.76 (95% CI: 6.51–11.54) months, respectively. The objective response rate was 36.3%, and the disease control rate was 80.3%, with no confirmed complete responses among 66 patients with measurable disease.

Clinical outcomes according to baseline NLR

To examine the prognostic value of NLR in the context of ramucirumab plus paclitaxel therapy, we subdivided the study patients according to a cut-off NLR of 2.94, which best segregated PFS outcomes into a high NLR group ($NLR \geq 2.94$, $n=31$) and a low NLR group ($NLR < 2.94$, $n=85$). The high NLR group had a significantly shorter TTP with first-line systemic therapy than the low NLR group (median: 4.2 vs. 7.9 months, respectively, $P < 0.001$).

Table 1 Baseline characteristics of study patients

Characteristics	Full cohort (n=116)	NLR<2.94 (n=85)	NLR≥2.94 (n=31)	P*
Age (year) [median (IQR)]	58 (47–63)	57 (47–63)	58 (49–63)	0.689
Male gender [n (%)]	71 (61.2)	51 (60.0)	20 (64.5)	0.821
ECOG PS [n (%)]				0.764
0–1	108 (93.1)	80 (94.1)	28 (90.3)	
2	8 (6.9)	5 (5.9)	3 (9.7)	
First-line TTP (month) [median (IQR)]	6.5 (3.9–12.1)	7.9 (4.7–13.2)	4.2 (1.6–6.2)	<0.001
NLR [median (IQR)]	2.0 (1.4–3.0)	1.7 (1.2–2.1)	4.4 (3.6–5.4)	<0.001
Hemoglobin (g/dL) ($\bar{x}\pm s$)	11.3±1.7	11.6±1.7	10.6±1.6	0.008
LDH elevation [n (%)]**	36 (33.6)	24 (31.2)	12 (40.0)	0.522
mMDSC (count/ μ L) [median (IQR)]	154.8 (29.8–306.7)	123.9 (22.2–238.7)	255.2 (100.1–81.2)	0.001
gMDSC (count/ μ L) [median (IQR)]	1,953.1 (937.0–2,948.0)	1,480.0 (98.5–2,561.6)	2,508.0 (1,770.9–5,523.4)	0.001

IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance status; TTP, time-to-progression; NLR, neutrophil-to-lymphocyte ratio; LDH, lactate dehydrogenase; mMDSC, monocytic myeloid-derived suppressor cells; gMDSC, granulocytic myeloid-derived suppressor cells; *, P-values represent comparison between NLR<2.94 and NLR≥2.94 groups. **, Baseline LDH levels were available in 107 patients: 77 and 30 patients in NLR<2.94 and NLR≥2.94 groups, respectively.

The high NLR group also had a significantly lower mean hemoglobin concentration as well as lower median mMDSC and gMDSC count than the low NLR group; in contrast, the baseline LDH levels were comparable between the two groups (*Table 1*).

During a median follow-up duration of 12.1 months for surviving patients, the high NLR group had a significantly poorer PFS [median, 2.79 (95% CI: 1.71–3.52) months] than the low NLR group [median, 3.68 (95% CI: 3.55–5.29) months] ($P=0.011$; *Figure 1A*). The high NLR group also had a significantly poorer OS [median, 4.64 (95% CI: 3.48–11.54) months] than the low NLR group [median, 9.07 (95% CI: 7.56–13.32) months] ($P=0.002$; *Figure 1B*).

We next performed univariate and multivariate analyses of PFS and OS using the prognostic factors previously identified in the second-line setting (*Table 2*). In the univariate analysis, an NLR≥2.94, TTP<6 months with first-line systemic therapy, and a hemoglobin concentration <10 g/dL were associated with poorer PFS [hazard ratio (HR)=1.77, 95% CI: 1.13–2.77, $P=0.012$; HR=2.11, 95% CI: 1.39–3.20, $P<0.001$ and HR=1.67, 95% CI: 1.05–2.67, $P=0.031$, respectively], while an NLR≥2.94, TTP <6 months with first-line therapy and a hemoglobin concentration <10 g/dL were associated with poorer OS (HR=2.18, 95% CI: 1.32–3.60, $P=0.002$; HR=2.21, 95% CI: 1.38–3.54, $P=0.001$ and HR=2.05, 95% CI: 1.19–3.53, $P=0.010$ respectively). Multivariate analyses revealed that both an NLR≥2.94 and TTP<6 months were independently associated with shorter PFS (HR=1.58, 95%

CI: 1.01–2.49, $P=0.046$ and HR=1.99, 95% CI: 1.31–3.05, $P=0.001$, respectively) and OS (HR=1.77, 95% CI: 1.04–3.04, $P=0.036$ and HR=1.99, 95% CI: 1.20–3.29, $P=0.008$, respectively). Additionally, an ECOG performance status of 2 was independently associated with shorter OS (HR=1.56, 95% CI: 1.04–2.34, $P=0.034$). Notably, neither the mMDSC count nor the gMDSC count was associated with PFS and OS in either the univariate or multivariate analysis.

Kinetics of MDSC counts and NLR after ramucirumab plus paclitaxel therapy

Uncertainty regarding the clinical value of the baseline mMDSC and gMDSC counts led us to further examine whether kinetics of MDSC counts following ramucirumab plus paclitaxel therapy would be associated with clinical outcomes. Although the mMDSC count did not change significantly (*Figure 2A*, left column), the gMDSC counts were significantly reduced after two cycles of ramucirumab plus paclitaxel therapy (*Figure 2B*, left column). These kinetic changes were further analyzed according to the occurrence of disease progression after two cycles of therapy. Again, the mMDSC count kinetics did not change significantly, regardless of disease progression (*Figure 2B*, right columns). In contrast, although the gMDSC count decreased significantly in patients who did not experience disease progression after two cycles of therapy, the gMDSC count tended to increase in patients with progressive disease after the second cycle (*Figure 2B*, right columns).

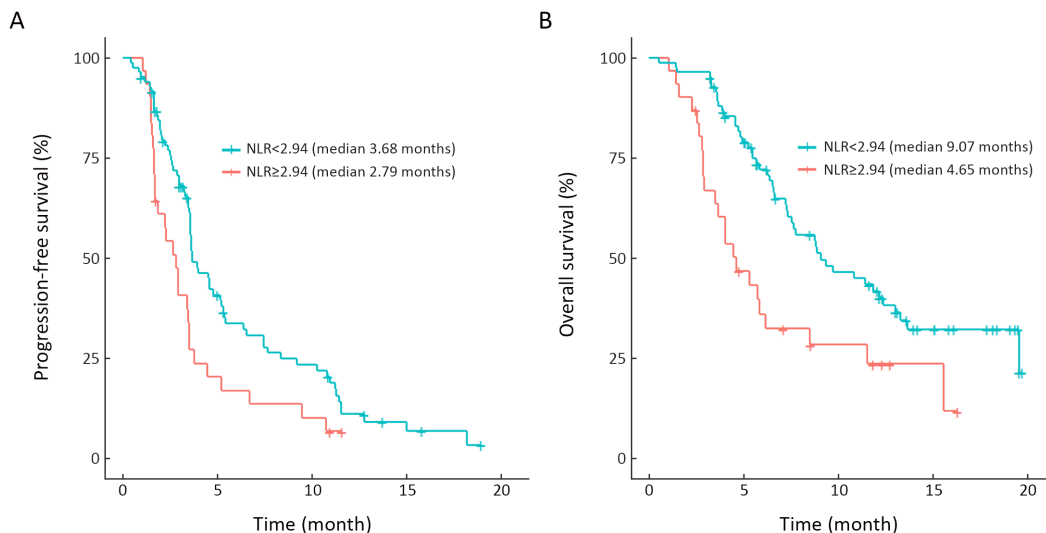


Figure 1 Survival outcomes according to baseline neutrophil-to-lymphocyte ratio (NLR). (A) Progression-free survival (P=0.011); (B) overall survival (P=0.002).

Table 2 Factors associated with PFS and OS

Variables	PFS				OS			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
NLR ≥ 2.94	1.77 (1.13–2.77)	0.012	1.58 (1.01–2.49)	0.046	2.18 (1.32–3.60)	0.002	1.77 (1.04–3.04)	0.036
Age	1.00 (0.98–1.02)	0.903	–	–	0.99 (0.97–1.02)	0.610	0.98 (0.96–1.01)	0.136
Male gender	0.93 (0.62–1.41)	0.735	–	–	0.77 (0.48–1.23)	0.278	–	–
ECOG PS 2	1.12 (0.76–1.65)	0.558	–	–	1.46 (0.98–2.18)	0.062	1.56 (1.04–2.34)	0.034
First-line TTP < 6 months	2.11 (1.39–3.20)	<0.001	1.99 (1.31–3.05)	0.001	2.21 (1.38–3.54)	0.001	1.99 (1.20–3.29)	0.008
Hemoglobin < 10 g/dL	1.67 (1.05–2.67)	0.031	–	–	2.05 (1.19–3.53)	0.010	1.74 (0.98–3.08)	0.057
gMDSC count	1.00 (0.99–1.00)	0.705	–	–	1.00 (1.00–1.00)	0.128	–	–
mMDSC count	1.00 (0.99–1.00)	0.367	–	–	1.00 (1.00–1.00)	0.044	–	–

PFS, progression-free survival; OS, overall survival; NLR, neutrophil-to-lymphocyte ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; TTP, time-to-progression; gMDSC, granulocytic myeloid-derived suppressor cell; mMDSC, monocytic myeloid-derived suppressor cell; 95% CI, 95% confidence interval; HR, hazard ratio.

NLR value also decreased significantly when taking the entire cohort into account (Figure 2C, left column); it also decreased among patients whose disease did not progress after two cycles of therapy (Figure 2C, right columns). However, NLR did not decrease among patients with early disease progression (Figure 2C, right columns). The similar patterns of change in gMDSC and NLR were supported by a positive correlation between gMDSC count and NLR at baseline and after two cycles of ramucirumab plus paclitaxel therapy (Supplementary Figure S2A). Sensitivity analysis showed that correlation between NLR and gMDSC counts was still significant even after exclusion of some influence points (Supplementary Figure S2B).

Clinical outcomes according to changes in gMDSCs

The opposing trends in gMDSC kinetics according to the occurrence of disease progression after cycle 2 led us to assume that an insufficient reduction or progressive increase in gMDSC count may be associated with the clinical outcomes. The percent changes in gMDSC counts are presented in Supplementary Figure S3, and a gMDSC percent increase ≥44% was identified as a hotspot cut-off for a conceptual comparison. Clinical characteristics of patient subgroups subdivided according to this criterion were comparable (Supplementary Table S1). However, patients with gMDSC percentage increases ≥44% had a

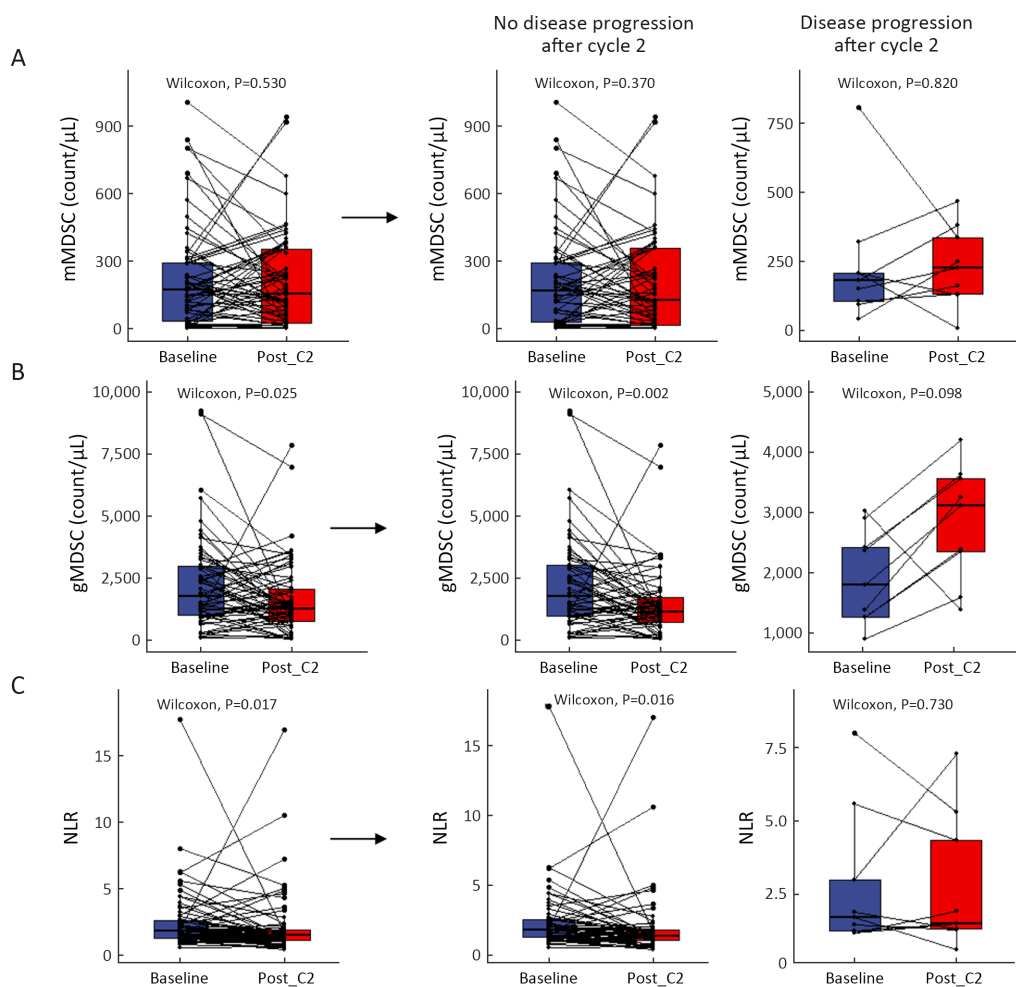


Figure 2 Kinetics of myeloid-derived suppressor cell (MDSC) counts and neutrophil-to-lymphocyte ratio (NLR) after ramucirumab plus paclitaxel therapy. (A) Kinetics of monocytic MDSC (mMDSC) counts; (B) granulocyte MDSC (gMDSC) counts; (C) NLR. The left column indicates overall changes, while the right columns indicate changes in subgroups with and without disease progression after two cycles of chemotherapy (Post_C2, post cycle 2).

significantly shorter median PFS of 2.96 (95% CI: 2.00–4.50) months than those with gMDSC percentage increases <44% [median PFS of 5.16 (95% CI: 3.68–7.66) months] ($P=0.001$; *Figure 3A*). Patients with gMDSC percentage increase $\geq 44\%$ also had poorer OS of median 6.51 (95% CI: 4.70–9.73) months than those with gMDSC percentage increases <44% [median OS of 13.05 months (95% CI: 7.76–NA) months] ($P=0.003$; *Figure 3B*).

To exclude the possibility that a higher degree of myelosuppression reflected a reduced gMDSC count after ramucirumab plus paclitaxel therapy, we compared the survival outcomes of subgroups with absolute neutrophil counts (ANCs) $\geq 1,000/\mu\text{L}$ and $\geq 1,500/\mu\text{L}$ after cycle 2. Among patients with post-treatment ANCs $\geq 1,000/\mu\text{L}$ or \geq

$1,500/\mu\text{L}$, those with a gMDSC percentage increase $\geq 44\%$ also had shorter median PFS and OS durations (PFS, $P<0.001$ and OS, $P=0.020$; PFS, $P=0.001$ and OS, $P=0.015$; *Supplementary Figure S4*, respectively).

When the therapeutic response to ramucirumab plus paclitaxel was compared according to the gMDSC kinetics, patients with gMDSC percentage increases $\geq 44\%$ had a higher proportion of progressive disease (30.7% vs. 0%, $P=0.033$ for measurable disease and 50% vs. 10%, $P=0.009$ for non-measurable disease; *Table 3*).

Discussion

In this study, we investigated the clinical value of NLR and

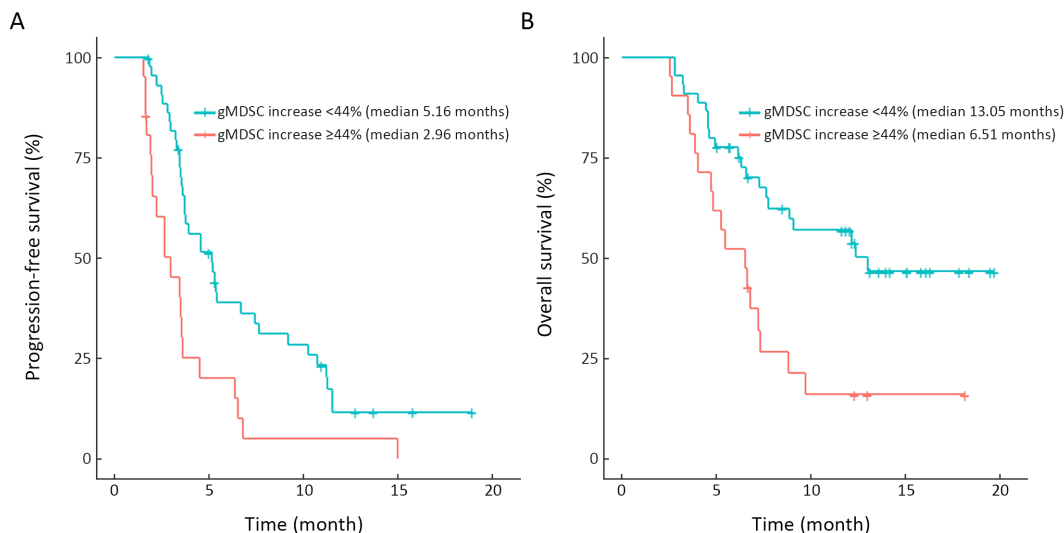


Figure 3 Survival outcomes according to percent increase in granulocyte myeloid-derived suppressor cell (gMDSC) count following ramucirumab plus paclitaxel therapy. (A) Progression-free survival (P=0.001); (B) overall survival (P=0.003).

Table 3 Response to ramucirumab plus paclitaxel according to gMDSC kinetics

Disease status	n (%)		P
	gMDSC increase <44%	gMDSC increase ≥44%	
Measurable disease			0.033
PR+SD	24 (100)	8 (66.7)	
PD	0 (0)	4 (33.3)	
Non-measurable disease			0.009
Non-CR/non-PD	18 (90.0)	4 (50.0)	
PD	2 (10.0)	4 (50.0)	

gMDSC, granulocytic myeloid-derived suppressor cell; PR, partial response; SD, stable disease; PD, progressive disease; CR, complete remission.

kinetics of MDSCs in patients with gastric cancer who received second-line ramucirumab plus paclitaxel treatment. Notably, NLR was independently associated with survival outcomes in our analysis. The overall gMDSC counts decreased after two cycles of ramucirumab plus paclitaxel therapy, but patients with progressive disease after cycle 2 tended to exhibit increasing gMDSC counts. We also found that a progressive increase in MDSC count was correlated with poor clinical outcomes. Our study appears to validate the prognostic value of baseline NLR in patients with gastric cancer who receive ramucirumab plus paclitaxel treatment. In addition, our study is the first to focus on the dynamic changes in MDSC populations and NLR of patients with gastric cancer in a therapeutic setting.

In our cohort, ramucirumab plus paclitaxel treatment yielded numerically shorter median PFS and OS durations (3.35 and 7.76 months, respectively) than those previously reported in the Expanded Access Program (17) (3.8 and 8.6 months, respectively) and the Rainbow trial (5) (4.4 and 9.6 months, respectively). When considered together with the higher percentage of patients with an ECOG performance status 2 in our study (6.9% vs. 3.1%) (17) and 0% (5), these decreases in survival may reflect the application of this regimen to a wider range of patients in routine practice.

The prognostic value of NLR was previously examined in patients with gastric cancer who received second-line paclitaxel (16) and ramucirumab with or without paclitaxel (17). However, the specific clinical value of NLR in patients receiving ramucirumab plus paclitaxel was not previously reported. In our study, we determined that along with a shorter TTP with first-line systemic therapy, a high NLR was independently associated with both PFS and OS in patients with gastric cancer who were treated with ramucirumab plus paclitaxel. Our results suggest that an “easily obtainable” baseline NLR may help guide clinical decisions in this clinical context. Notably, in addition to the prognostic implication of baseline NLR, we newly discovered that kinetic changes in NLR coincide with the patients’ clinical outcomes. The potential association of a high NLR with poor clinical outcomes in cancer patients may be explained by several mechanisms. Neutrophils, the core component of NLR value, can suppress T-cell function by producing arginase-1 and hydrogen peroxide (30,31). Circulating factors implicated in cancer

progression, such as granulocyte colony-stimulating factor (32,33) and VEGF (20,24), were also shown to be associated with the NLR. Additionally, cancer patients with high NLRs may also have elevated MDSC counts (19,20,34). However, our data suggest that baseline MDSC count appears to have no additional prognostic value than the NLR, which discourages the use of MDSC count as a prognostic factor. The exact mechanism by which NLR is correlated with the clinical outcomes of patients with gastric cancer requires further elucidation.

Examination of kinetics of MDSC counts before and after ramucirumab plus paclitaxel therapy revealed an overall decrease in gMDSC counts after treatment. This is consistent with preclinical data that demonstrated the potential effects of ramucirumab and paclitaxel on the depletion of MDSCs or differentiation of these cells into dendritic cells (25-27). Notably, opposing trends in gMDSC kinetics were observed according to the occurrence of disease progression after two cycles of therapy, and similar patterns were observed in NLR kinetics. Together with the association between changes in gMDSC counts and clinical outcomes, these results suggest the potential implications of dynamic changes of MDSCs in this clinical setting. The observed differential clinical outcomes according to gMDSC changes are in line with previous studies that highlighted the clinical value of dynamic changes in immune subsets (35-37). However, one critical consideration is the remaining uncertainty regarding whether a progressive increase in gMDSCs directly contributes to tumor progression or is itself a bystander phenomenon driven by other crucial factors. Nevertheless, our data raise an important question regarding the delineation of critical factors that contribute to disease progression in association with the dynamic changes of gMDSC counts. Future studies should aim to validate the clinical relevance and elucidate the mechanism underlying our findings. In particular, the identification of baseline factors associated with differential immunomodulatory effects of ramucirumab plus paclitaxel treatment will be clinically important.

Reductions in the proportions of MDSCs after systemic chemotherapy regimens including bevacizumab or paclitaxel have been reported in other types of cancer. In patients with non-small cell lung cancer, bevacizumab-containing chemotherapy regimens reduced the proportion of gMDSCs, in contrast to non-bevacizumab regimens (38). Significant reductions in the MDSC counts were also reported in patients with breast cancer after treatment with

paclitaxel (39). Limagne *et al.* also reported that a proportional decrease in gMDSC count was associated with favorable survival outcomes in colorectal cancer patients who received bevacizumab-based FOLFOX chemotherapy (40). However, when evaluating a reduction in the MDSC count, one should consider that variations in the degree of myelosuppression can be related to different MDSC counts after systemic chemotherapy, which was not addressed by these previous studies. To avoid this potential bias, we performed subgroup analyses of patients with preserved ANC counts and achieved consistent outcomes. Future studies that address changes in the MDSC counts should consider this aspect.

Conclusions

Our results suggest that NLR is an independent prognostic factor in patients with gastric cancer who are receiving second-line ramucirumab plus paclitaxel treatment. NLR may help guide clinical decisions in this clinical setting. The association between an increase in the gMDSC count and a poor clinical outcome raises an important issue regarding further investigations of mechanism and validation of clinical relevance of MDSC kinetics in gastric cancer patients.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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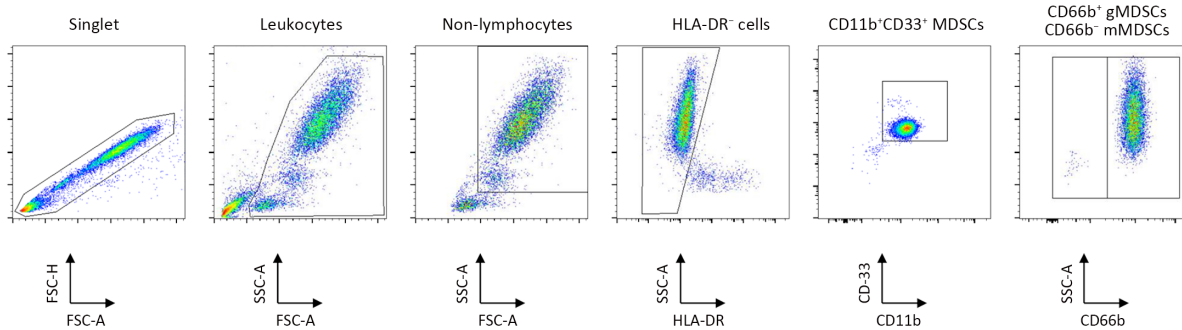


Figure S1 Gating strategy of flowcytometry analysis of MDSCs. MDSC: myeloid-derived suppressor cell; gMDSC, granulocyte MDSC; mMDSC, monocytic MDSC.

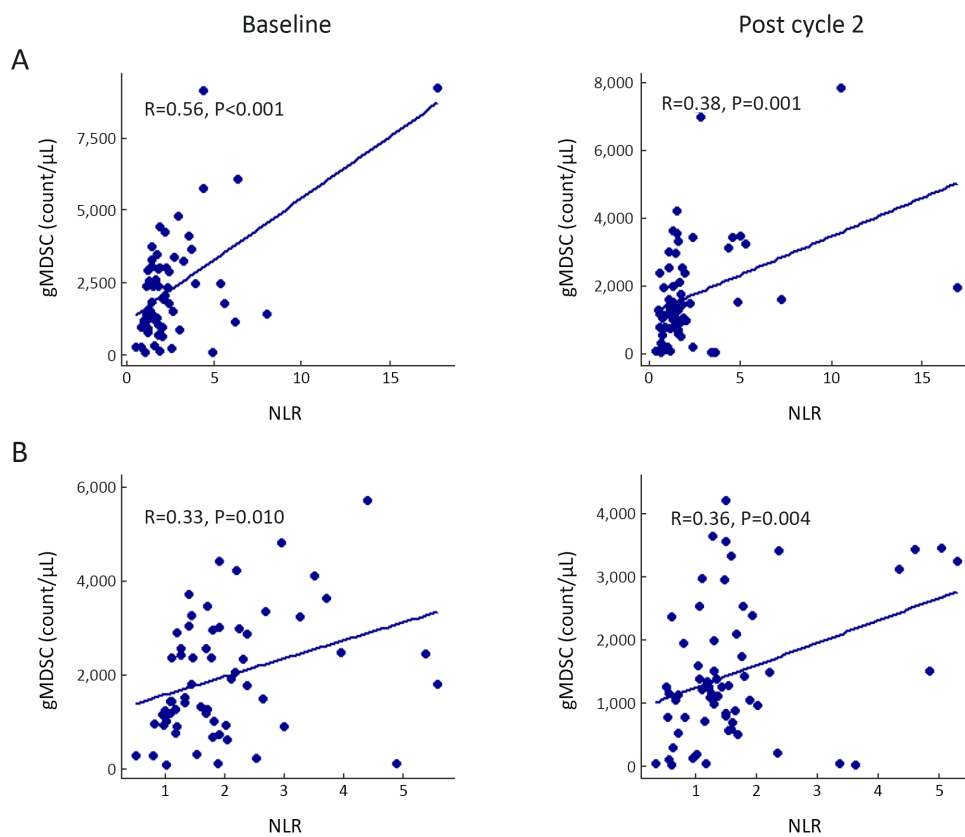


Figure S2 Correlation between NLR and gMDSC counts at baseline and after two cycles of ramucirumab plus paclitaxel therapy. (A) Full cohort; (B) After exclusion of some influence points. NLR, neutrophil-to-lymphocyte ratio; gMDSC, granulocyte myeloid-derived suppressor cell.

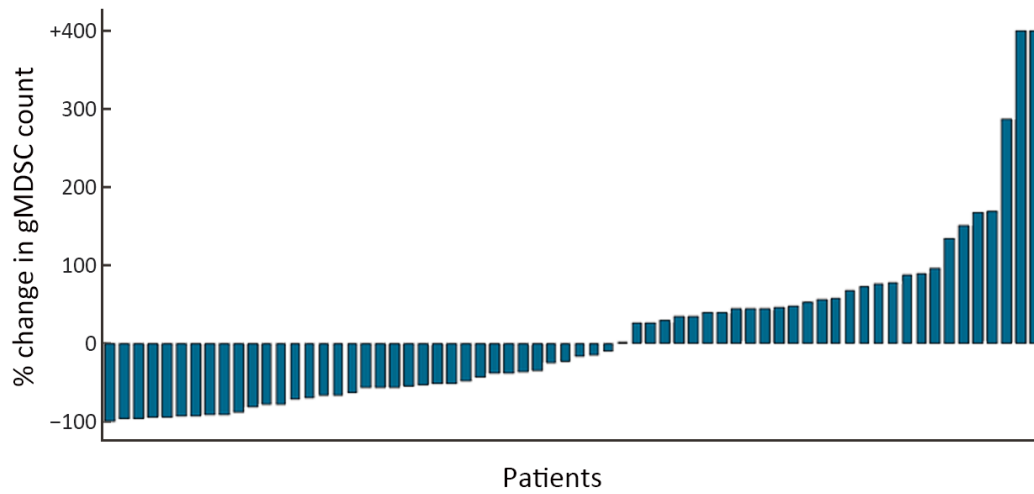


Figure S3 Percent changes in gMDSC counts from baseline to after two cycles of ramucirumab plus paclitaxel therapy. gMDSC, granulocyte myeloid-derived suppressor cell.

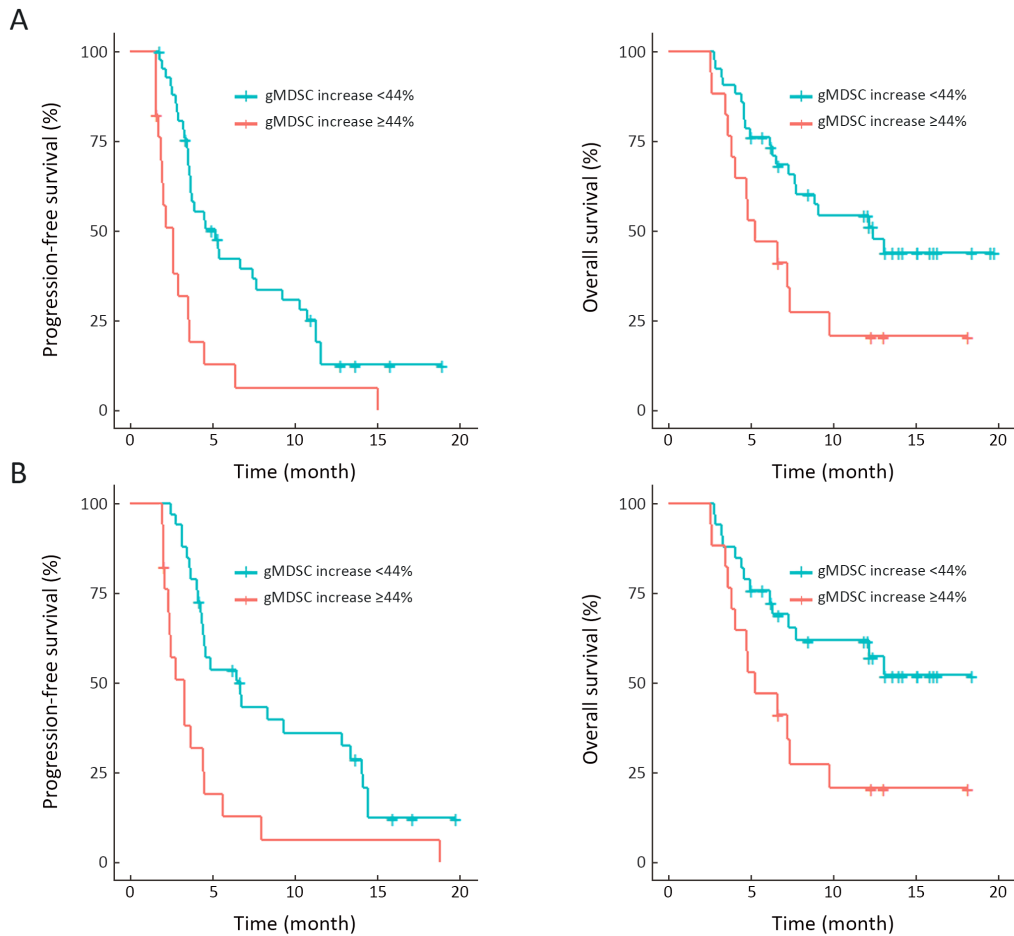


Figure S4 Survival outcomes according to percent increase in gMDSC count following ramucirumab plus paclitaxel therapy. (A) $ANC \geq 1,000/\mu L$ (PFS, $P < 0.001$; OS, $P = 0.020$) and (B) $ANC \geq 1,500/\mu L$ (PFS, $P = 0.001$; OS, $P = 0.015$) after cycle 2 of ramucirumab plus paclitaxel therapy. gMDSC, granulocyte myeloid-derived suppressor cell; ANC, absolute neutrophil counts; PFS, progress-free survival; OS overall survival.

Table S1 Clinical characteristics according to percent change of gMDSC count

Variables	gMDSC increase <44% (n=45)	gMDSC increase ≥44% (n=21)	P
Age (year) ($\bar{x}\pm s$)	54.5±10.9	54.2±11.9	0.943
Male gender [n (%)]	30 (66.7)	11 (52.3)	0.535
ECOG PS [n (%)]			0.763
0–1	43 (95.6)	19 (90.5)	
2	2 (4.4)	2 (9.5)	
TTP with first-line therapy (month) [median (IQR)]	6.6 (4.7–10.9)	5.7 (3.2–12.8)	0.334
NLR [median (IQR)]	1.8 (1.3–2.7)	1.9 (1.2–2.5)	0.983
Hemoglobin (g/dL) ($\bar{x}\pm s$)	11.7±1.4	11.4±1.9	0.426
LDH (IU/L) [median (IQR)]	212.0 (182.5–251.5)	194.0 (187.0–240.0)	0.776

gMDSC, granulocytic myeloid-derived suppressor cell; ECOG PS, Eastern Cooperative Oncology Group performance status; TTP, time-to-progression; NLR, neutrophil-to-lymphocyte ratio; LDH, lactate dehydrogenase.